إحكام رقابة الجودة على المستحضرات الصيدلانية المحلية والمستوردة خلال موسم الحج والعمرة للعام 1425 ه

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ملخص البحث

نظرا لأهمية تداول الدواء بين ضيوف الرحمن وأهالي العاصمة المقدسة بجرعاته وتراكيزه المضبوطة أثناء فترة العلاج. فمن الحكمة إحكام ورقابة الجودة علي هذه المستحضرات الدوائية المتواجدة بالأسواق سواء كانت محلية أو مستوردة وذلك باستحداث وتطبيق طرق لونية وطيفية جديدة ومبتكرة وكذا مقارنتها إحصائيا بالطرق الدستورية المعتمدة. وتعتمد هذه الطرق على تكون متراكبات المزدوج الأيوني مع الأدوية المختارة من مشتقات السيفالوسبورن. هذه المتراكبات اللونية لها خصائص حساسة وخصوصية كبيرة في التفاعل وأعطت أعلى طول موجي عند 521 و566 نانومتر وتم تحديد المدى التركيزي للتقدير من 2 وحتى 68 ميكروجرام لكل مللي وطبقت الطريقة أولا علي المادة الخام النقية وأعطت نتائج مشجعة جاد عند مقارنتها بالطرق الدستورية المعتمدة ويتراوح نسبة الكفاءة عند 99.85 %. وعند تطبيق الطرق المقترحة علي المستحضرات الدوائية المتداولة أثناء موسم الحج للعام 1425 ه وجد أن الطرق المقترحة علاوة علي الطرق الدستورية تعطي نتائج متقاربة جدا من الناحية الإحصائية ولكنها تختلف عن التراكيز المدونة علي العينات نفسها بنسب تتراوح بين 3.0 و10.0 % وذلك لأحد السببين التاليين أو كليهما معا:

الأول: وهو عملية التصنيع والإنتاج وعدم الرقابة أثناء التصنيع.

الثاني: وهو الأرجح أن تكون عمليات التخزين والتهوية غير مطابقة للمواصفات وخاصة وان نسب الكفاءة والخطأ تقل مع حداثة تاريخ الإنتاج وتزداد كما ابتعد الإنتاج وللتأكد تم العمل علي عينة باقي علي نهاية الصلاحية لهل ستة أشهر ووجد بما لا يدع مجال للشك بالطرق المقترحة وكذلك الدستورية أن نسبة المادة الفعلة لا تزيد عن 83 % وان نسبة الخطأ تجاورت ال16 % مع ملاحظة مقارنة الدقة والكفاءة بين الطرق والتي أظهرت تماثلا وتكاملا واضحا عند مقارنتها إحصائيا.

إن أهم توصيات البحث هو خضوع عمليات التخزين والنقل والتعبئة والتهوية للمواصفات القياسية المتبعة في تلك الصناعة حتى يؤتي الدواء ثماره المرجوة منه ويساعد في شفاء ضيوف الرحمن وأهالي العاصمة المقدسة بأمر وإذن الله.

Quality control for pharmaceutical formulations during hajj sezon (1425 H) containing certain cephalosporins

Abstract

To evaluate the quality of pharmaceutical formulations containing certain cephalosporins during hajj sezon (1425 H), a simple and reproducible spectrophotometeric method for the assay of cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium with methyl red and rose bengal reagents has been developed. The procedure is based on ion pair complex formation in buffer medium of pH 5.6 and 9.00 , respectively. Beer's law is obeyed in the range 2.0-68 μg ml^{-1} at λ_{max} 521 and 566 nm using methyl red and rose Bengal, respectively. For more accurate analysis, Ringbom optimum concentration range is found to be 1.8 - 65 μg ml^{-1}. The molar absorptivity and Sandell sensitivity were calculated. Six replicate analysis of solutions containing seven different concentrations of the examined drugs were carried out and gave a mean correlation coefficient ≤ 0.9988; the factors of the regression line equation for the three cephalosporins were calculated. The proposed method was applied to the determination of the examined drugs in pharmaceutical formulations and the results demonstrated that the method is equally accurate, precise and reproducible as the official methods.

1. Introduction

Cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium are some of the third-generation cephalosporin antibiotics

characterized by a broad antibacterial spectrum and a resistance to beta-lactamase-producing organisms. In addition to its antimicrobial activity [1], cephalosporins are distributed widely into tissues and body fluids, including pleural, pericardial and synovial fluids. However, while the earlier cephalosporins failed to penetrate the central nervous system and were unsuccessful in the treatment of meningitis, the third-generation cephalosporins enter the central nervous system and reach therapeutic concentrations there, sufficiently for treatment of meningitis caused by aerobic gram-negative bacteria [2]. These characteristics are of considerable clinical and hence, analytical interest [3].

During hajj sezon, many humans take different antibiotics with variable dose. Therefore, we aimed to evaluate the quality of the pharmaceutical formulations used in this sezon by developing a simple colorimetric assay in the field and comparing the obtained results with the official methods used for that purposes.

Several analytical procedures are available in the literature for the analysis of cephalosporins, viz spectrophotometric [4-10], polarographic [11], stripping voltammetric [11-13], fluorimetric [14,15] and high performance liquid chromatographic [16-18] methods.

The aim of this work was to develop a simple and reproducible colorimetric procedure for the determination of cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium in acidic medium by ion pair chemical reaction with methyl red and rose Bengal as counter ions.

2. Experimental

2.1. Material and reagents

All chemicals and reagents used were of analytical grade and all solutions were prepared in double distilled water.

A freshly prepared $5x10^{-3}$ M aqueous solution of methyl red and rose Bengal was prepared by dissolving appropriate weight in warm water. Buffer solution [19], a mixture of 250 ml of 0.2 M potassium hydrogen phthalate and different volume of 0.1 M HCl was diluted to one liter with water to produce different pH values.

Cefotaxime sodium (I) was obtained from Hoechst Orients Egypt, Cairo, under the license from Hoechst AG, Frankfurt/Main, Germany, whereas cefuroxime sodium (II) was obtained from Glaxo Wellcome, Egypt, S.A.E., Cairo, under the license from Glaxo Wellcome group Ltd., England. Ceftriaxone disodium (III) was obtained from the Egyptian International Pharmaceutical Industries Company (EIPICO) under the license from Roche (Switzerland). Stock solutions were prepared by accurately weighing 100 mg of the examined drug into a 100 ml calibrated flask, dissolved in warm water and kept in the dark to avoid any degradation of the drugs.

The following commercial formulations were subjected to the analytical procedure, claforan vials (Hoechst Orient Egypt, Cairo) containing 524 mg cefotaxime sodium equivalent to 500 mg cefotaxime per vial, Claforan vials (Laboratorires Roussel 97, rue-de Vaugrirared- Paris) containing 524 mg cefotaxime sodium equivalent to 500 mg cefotaxime per vial, and 1048 mg cefotaxime sodium equivalent to 1000 mg cefotaxime per vial, Primocef (Julphar, Gulf

Pharmaceutical Industries, Ras Al-Khaimah, U.A.E.) Containing 250, 500, 1000 and 2000 mg cefotaxime sodium per vial, zinnat vials (Glaxo Wellcome, Egypt) containing 263 mg, equivalent to 250 mg cefuroxime and rocephen vials (EIPICO, Egypt) containing ceftriaxone disodium equivalent to 1.0 g ceftriaxone per vial, rocephen vials (Roche, F. Hoffmann-La-Roche Ltd, Basel) containing ceftriaxone disodium equivalent to 500, and 1000 mg ceftriaxone per vial were used.

2.2. Instrumentation

Spectral and absorbance measurements were made with Perkin-Elmer Lambda 5B spectrophotometer UV / Vis with 10-mm quartz cells (New York, USA). The pH of solutions was checked using an Orion Research Model 601A/ digital ionlyser (New York, USA).

2.3. General Procedure

Pipette a 1.5 ml aliquot of the examined drug solution (concentration range as indicated in Table 1) in a 10 ml calibrated flask. Add 3.0 ml of buffer solution of pH 5.6 and 9.0 using methyl red and rose bengal, respectively, 1.0 ml of 5×10^{-3} M reagent solution (freshly prepared) and 2.0 ml of ethanol. Allow the mixture to stand at 50 ± 2 °C for 5.0 min and then dilute to volume with water. Measure the absorbance at 520 and 566 nm using methyl red and rose bengal, respectively, against a reagent blank prepared in a similar manner. The examined drug concentration was read from a standard calibration curve prepared with the same manner under identical conditions without the examined drug.

2.4. Procedure for vials

The contents of each vial was transferred into separate 500-ml calibrated flask and made up to volume with water. Suitable aliquots of the standard drug solutions were mixed with 0.5 ml of the solution prepared above in 50 ml calibrated flask and diluted to the mark with water. The assay was completed as described above under general procedure. The recovery of the drug was computed from the corresponding regression equation.

2.5. Stoichiometric relationship

Job's method of continuous variation was employed; a 5×10^{-3} M standard solution of drug (cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium and 5×10^{-3} M solution of reagent (methyl red and rose bengal) were used. A series of solution were prepared in which the total volume of drug and reagent was kept at 2.0 ml. The reagents were mixed in various proportions and diluted to volume in a 10 ml calibrated flask with the appropriate solvent following the above mentioned procedures.

3. Results and discussion

Preliminary investigations revealed that cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium react with each of reagents methyl red and rose Bengal to yield soluble ion pair complexes exhibiting absorption maximal at 521, and 566 nm using methyl red and rose Bengal, respectively. Under the experimental conditions, the corresponding reagent blank showed a negligible

absorbance. Investigations were carried out to establish the most favourable conditions for ion-pair complexation reaction of reagents (methyl red and rose Bengal) with the studied drugs to achieve maximum colour development in their determination. The influence of some variables on the reaction has been tested as follows.

3.1. Effect of pH

In order to establish the optimum buffer media, different buffers were examined as acetate, borate, thiel, phosphate and universal buffers. It was noticed that the maximum colour intensity and constant absorbance were found using phosphate buffer solutions of pH 2.0 - 12.50. Highly and constant absorbances were obtained over the pH range 5.10- 6.20 and 8.60 – 9.50 using methyl red and rose Bengal, respectively, as represented in Figs. (1,2). Moreover the volume of the optimum pH value was examined and found to be 3.0 ml in the total volume of 10 ml.

3.2. Effect of reagent concentration

The influence of reagents (methyl red and rose Bengal) concentration on the absorbance of the ion-pair is investigated. Net absorbance increases with reagent concentration and the optimum values are obtained for the concentrations between 5.0×10^{-4} and 4.0×10^{-4} M, respectively. An increase in reagent concentration causes a decrease in absorbance because of an increase in the absorbance of the blank. Hence, 5.0×10^{-4} M in the final assay solution was selected for the general procedure, since the results are highly concordant at this level of concentration (Figs. 3, 4).

3.3. Effect of time and temperature

Sample solutions containing drug and the blank were treated identically with the reagent and buffer within different time and temperature. The results obtained indicated that ion-pairs were formed after 5.0 min of starting the reaction at room temperature (25 ± 1°C). The absorption spectra were not altered on varying temperature upto 60 °C, after which the absorbance decay with 20 % for each 5 °C raising. The absorbance remained stable at room temperature for at least 18 h, after which it began to fade slowly.

3.4. Composition of the complex

The stoichiometry of the complexes formed between cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium, with reagents (methyl red and rose Bengal) was investigated at the optimum pH value applying the molar ratio and continuous variation methods. The results indicated the formation of 1:1 ion-pair complexes. The logarithmic stability constants was calculated and recorded in Table 1. The presence of the ion pair complexes may be supported by the bathochromic shift observed from 485 nm for methyl orange reagent to 521, 520 and 522 nm for cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium, using rose bengal reagent, a bathochromic shift from 522 nm to 566, 567 and 565 nm, respectively, for the above mentioned drug.

3.5. Analytical features

Regression plots showed that there was a linear dependence of absorbance on concentration over the Beer's law ranges. The optimum

conditions were those used in the procedure. The molar absorptivity, Sandell sensitivity, slope, intercept, correlation coefficient, detection and quantification limits were obtained by a linear least-squares treatment of the results for ion pairs in solution. For more accurate analysis, Ringbom optimum concentration ranges were calculated (Table 1).

The reproducibility of the proposed procedure was determined by analyzing ten replicate samples of each drug (40 μg ml^{-1}). The relative standard deviations and ranges of error obtained are given in Table 1.

The performance of the proposed procedure was assessed by calculation of the t- (for accuracy) and F- (for precision) values compared with the official methods [20]. Mean values were obtained in a Student's t- and F- tests and 95% confidence limits for five degrees of freedom [21] and the results showed that the calculated t- and F- values did not exceed the theoretical values (Table 1).

In order to determine the accuracy and precision of the proposed procedure, solutions containing four different concentrations of the examined drugs were prepared and analysed in quantuplicate. The measured standard deviation, relative standard deviation (S_r), the standard analytical error and confidence limit value (Table 2) can be considered satisfactory, at least for the levels of concentrations examined.

The standard deviation of the absorbance measurements was obtained from a series of 13 blank solutions. The detection (k=3) and quantitation (k=10) limits of the method were established according to the IUPAC definitions ($C_l = KS_0/s$) where C_l is the detection limit, S_0

is the standard error of blank determination, s is the slope of the standard curve and K is the constant related to the confidence interval [22]. The calculated values for detection and quantitation limits were recorded in Table (1).

Comparison of the recovery obtained with the proposed method with the purity of the examined drugs as determined according to the official method [20] showed that a high accuracy of the present method. The proposed method is simpler, rapid and more sensitive than the official method [20]. Moreover, the proposed method could be used for the routine determination of drug cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium in pure form or in pharmaceutical formulations.

3.6. Interference studies

The effects of common excipients that often accompany the studied drugs in various pharmaceutical dosage forms were tested for possible interference in the assay. An attractive feature of the procedure is its relative freedom from interference by the usual tablet diluents and excipients such as talc, sucrose, starch, gelatin, lactose and magnesium stearate. Amounts far in excess of their normal occurrences in dosage forms were added, and no effect due to these excipients was noted in the experimental procedure. Analyzing synthetic mixtures of the drugs (mg) containing the following amounts of excipients (mg) also checked the applicability of the method:

1. cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium (20), talc (60), sucrose (40), starch (50), gelatin (50), lactose (30) and magnesium stearate (60).

2. cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium (20), talc (60), sucrose (30), starch (40), gelatin (50), lactose (30) and magnesium stearate (50).

3. cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium (20), talc (40), sucrose (35), starch (60), gelatin (50), lactose (35) and magnesium stearate (60).

A Suitable amount of each synthetic mixture was analyzed by following the general procedure described earlier. The percentage recovery was found to be in the range of 98.9 – 100.8 with RSD values $\geq$ 1.25 for five replicates. Moreover, there is no interference from the decarboxylated degradant results from thermal and hydrolytic treatment.

3.7. Analytical applications

The proposed procedure was further applied to the analysis of certain dosage forms during hajj sezon (1425 H) containing cefotaxime sodium, cefuroxime sodium and ceftriaxone disodium under consideration. The results in Table 3 are in accordance with those obtained by the official methods [20].

Statistical analysis [21,22] of the results using Student t- test and the variance ratio F- test showed no significant difference between the performance of the proposed and official methods as regards to accuracy and precision.

4. Conclusion

The proposed procedure is fairly simple, less time-consuming and more sensitive than the official methods [21]. The principal advantage of the proposed procedure is suitable for the determination of the studied drugs in their dosage forms without interference from excipients and additives or from common degradation products suggesting application in bulk drug analysis. Statistical comparison for the results of the proposed procedure with the official methods indicates that there is no significant difference with regard to accuracy and precision. Applying the proposed methods on dosage forms used during hajj sezon (1425 H), indicated that some of dosage forms lose its activity for different reasons by 3.0 to 10% and the losing is proportional with data of production.

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Table 1: Quantitative parameters for the proposed procedure

Parameter	Methyl red			Rose bengalCongo red		
	I	II	III	I	II	III
pH	5.6	5.5	5.6	9.00	9.00	9.00
λ_{max}	521	520	522	566	567	565
Beer's conc. range /µg ml^{-1}	2.0–64.0	2.0–68.0	2.0– 59.0	2.0 –62.0	2.0 –60.0	2.0 –65.0
Ringbom conc. range / µg ml^{-1}	3.5-60.0	4.0 –65.0	4.08 – 56.5	3.0 –57.0	4.0 –55.0	3.0 –61.4
Detection limit /µg ml^{-1}	0.34	0.45	0.37	0.34	0.36	0.35
Quantification limit /µg ml^{-1}	1.98	2.08	2.02	1.97	2.05	1.90
Molar absorptivity / L mol^{-1} cm^{-1}	$8.32x10^4$	$5.3x10^4$	$2.97x10^4$	$3.21x10^4$	$2.83x10^4$	$3.29x10^4$
Sandell sensitivity / ng cm^{-2}	4.17	6.67	10.26	10.81	12.50	9.30
Stoichiometric ratio	1: 1	1: 1	1: 1	1: 1	1: 1	1: 1
Stability constant	5.69	5.89	5.50	9.06	8.64	9.65
Stability / h	15	15	18	15	18	18
Regression equation [a]						
Slope	0.14	0.09	0.10	0.09	0.08	0.11
Intercept	-0.037	0.056	0.040	0.067	-0.025	-0.030
Correlation coefficient (r)	0.9996	0.9992	0.9988	0.9994	0.9998	0.9996
RSD % of slope	$3.45x10^{-4}$	$6.13x10^{-4}$	$5.27x10^{-4}$	$7.32x10^{-4}$	$5.34x10^{-4}$	$8.09x10^{-4}$
RSD % of intercept	$1.67x10^{-4}$	$2.98x10^{-4}$	$2.67x10^{-4}$	$3.45x10^{-4}$	$2.65x10^{-4}$	$4.12x10^{-4}$
Range of error	1.20	1.15	1.45	1.00	1.35	1.50
RSD %	0.91	0.78	0.85	1.15	1.07	0.98
Student t- value (2.57)[b]	0.35	0.71	0.75	0.76	0.35	0.56
Variance ratio F-test (5.05)[b]	1.72	4.07	2.92	1.02	3.20	1.49

[a] A = a + bC, where C is the concentration in µg ml^{-1}

[b] Values in parentheses are the theoretical values for t- and F- values at 95% confidence limits and five degrees of freedom.

Table 2: Evaluation of accuracy and precision of the proposed method compared with that of the official one [20].

Drug	Taken μg ml^{-1}	Found* /μg ml^{-1}					
		Off.	Prop.	SD	RSD (%)	SAE	Confidence Limits
I	2.5	2.55	2.52	0.04	0.58	0.016	2.52 ± 0.050
	5.0	4.90	5.05	0.07	0.19	0.029	5.05 ± 0.080
	7.5	7.65	7.45	0.09	1.10	0.037	7.45 ± 0.110
	10.0	10.20	9.90	0.05	0.76	0.020	9.90 ± 0.060
	12.5	12.70	12.40	0.08	0.99	0.033	12.40 ± 0.095
	15.0	15.25	15.15	0.10	1.31	0.042	15.15 ± 0.120
	17.5	17.20	17.60	0.11	1.40	0.046	17.60 ± 0.135
II	3.0	2.95	3.03	0.05	0.64	0.020	3.03 ± 0.060
	6.0	6.10	5.95	0.03	0.41	0.012	5.95 ± 0.035
	9.0	9.15	8.90	0.08	0.96	0.033	8.90 ± 0.095
	12.0	11.80	12.10	0.06	0.75	0.024	12.10 ± 0.020
	15.0	14.80	15.10	0.09	1.15	0.037	15.10 ± 0.110
	18.0	18.20	17.85	0.07	0.97	0.029	17.85 ± 0.080
	20.0	20.30	19.85	0.12	1.45	0.140	19.85 ± 0.145
III	4.0	4.10	4.03	0.03	0.46	0.012	4.03 ± 0.035
	8.0	7.85	8.05	0.07	0.83	0.027	8.05 ± 0.080
	12.0	12.15	11.90	0.05	0.64	0.20	11.90 ± 0.060
	16.0	15.80	15.90	0.08	0.98	0.033	15.90 ± 0.095
	20.0	19.75	20.15	0.06	0.75	0.024	20.15 ± 0.070
	24.0	23.70	24.20	0.09	1.07	0.037	24.20 ± 0.110
	28.0	28.35	27.85	0.04	0.59	0.016	27.85 ± 0.050
Mean					0.76	0.024	

* Average of six determinations.

Table 3: Determination of cefotaxime sodium (I), cefuroxime sodium (II) and ceftriaxone disodium (III) in pharmaceutical preparations applying the standard addition technique.

Pharmaceutical Formulation	Taken μg ml^{-1}	Added μg ml^{-1}	Examined drug Found* μg ml^{-1}		
			Proposed (MR) ± SD	Proposed (MR) ± SD	Official ± SD
Zinnat (250 mg of II)	10	0	9.85 ± 0.53	9.80 ± 0.42	9.90 ± 0.75
		10	19.80 ± 0.40	19.75 ± 0.38	19.90 ± 0.99
		15	24.80 ± 0.53	24.70 ± 0.56	24.84 ± 0.81
		20	29.70 ± 0.64	29.60 ± 0.65	30.25 ± 1.03
		25	34.60 ± 0.88	34.50 ± 0.48	35.20 ± 1.17
		30	39.60 ± 0.58	39.50 ± 0.67	40.25 ± 1.34
Claforan (500 mg of I)	5.0	0	4. 84 ± 0.34	4.75 ± 0.32	4.85 ± 0.64
		10	14.80 ± 0.41	14.70 ± 0.45	14.85 ± 0.41
		20	24.70 ± 0.37	24.65 ± 0.57	24.65 ± 0.88
		30	34.70 ± 0.81	34.65 ± 0.43	34.90 ± 1.07
		40	44.50 ± 0.93	44.50 ± 0.60	45.10 ± 1.13
		50	54.50 ± 0.66	54.45 ± 0.44	54.60 ± 0.78
Claforan (500 mg of I)	7.5	0.0	7.25 ± 0.95	7.20 ± 0.67	7.15 ± 0.54
		15	22.15 ± 0.83	22.15 ± 0.78	22.05 ± 0.86
		30	37.05 ± 0.67	37.00 ± 0.92	37.00 ± 0.96
		45	51.80 ± 0.97	51.70 ± 1.08	51.60 ± 1.11
		52.2	59.10 ± 1.05	59.20 ± 0.94	59.15 ± 0.99
Primocef (500 mg of I)	5.0	0.0	4.80 ± 0.76	4.75 ± 0.58	4.75 ± 0.47
		7.5	12.25 ± 0.56	12.30 ± 0.46	12.20 ± 0.67
		15	19.70 ± 0.70	19.70 ± 0.68	19.65 ± 0.80
		22.5	26.60 ± 0.48	26.65 ± 0.62	26.70 ± 0.87
		30	34.50 ± 0.66	34.55 ± 0.43	34.60 ± 0.92
		37.5	41.95 ± 0.75	41.90 ± 0.71	42.00 ± 1.01
Primocef (1000 mg of I)	10	0.0	9.70 ± 0.85	9.65 ± 0.90	9.60 ± 0.97
		10	19.60 ± 0.93	19.65 ± 0.69	19.50 ± 0.79
		20	29.50 ± 1.01	29.60 ± 1.08	29.40 ± 1.13
		30	39.40 ± 1.10	39.40 ± 1.12	39.25 ± 1.05
		40	49.25 ± 0.90	49.30 ± 0.91	49.20 ± 0.86
		50	59.10 ± 0.88	59.15 ± 1.15	59.00 ± 1.09
Primocef (2000 mg of I)	15	0.0 ±	14.50 ± 1.12	14.55 ± 0.98	14.50 ± 0.86

		7.5 ±	22.00 ± 1.20	21.95 ± 1.05	21.90 ± 0.96
		15 ±	29.40 ± 1.07	29.35 ± 1.14	29.30 ± 1.25
		22.5 ±	31.80 ± 0.97	31.75 ± 1.11	31.75 ± 1.22
		30 ±	44.10 ± 0.89	44.15 ± 0.96	44.00 ± 1.30
		37.5 ±	51.55 ± 1.22	51.50 ± 1.17	51.40 ± 1.33
Rocephen (1000 mg of III)	20	0.0	19.45 ± 0.55	19.40 ± 0.75	19.25 ± 1.25
		7.5	26.90 ± 0.77	26.85 ± 0.87	26.75 ± 1.42
		15	24.30 ± 0.82	34.25 ± 0.96	34.35 ± 0.97
		22.5	41.60 ± 0.68	41.65 ± 0.95	41.50 ± 1.63
		30	48.85 ± 0.56	48.80 ± 1.05	48.75 ± 1.37
		37.5	56.60 ± 0.74	56.70 ± 0.66	56.55 ± 1.71
Rocephen (1000 mg of III)	15	0.0	14.50 ± 0.66	14.60 ± 0.85	14.50 ± 1.11
		10	24.45 ± 0.97	24.50 ± 0.78	24.40 ± 1.21
		20	34.40 ± 0.87	34.45 ± 0.98	34.30 ± 0.99
		30	44.25 ± 1.04	44.30 ± 1.14	44.30 ± 1.25
		40	44.20 ± 1.11	54.25 ± 0.97	54.15 ± 1.20
		50	64.00 ± 1.20	64.00 ± 1.23	64.05 ± 0.87

*Average of six determinations.

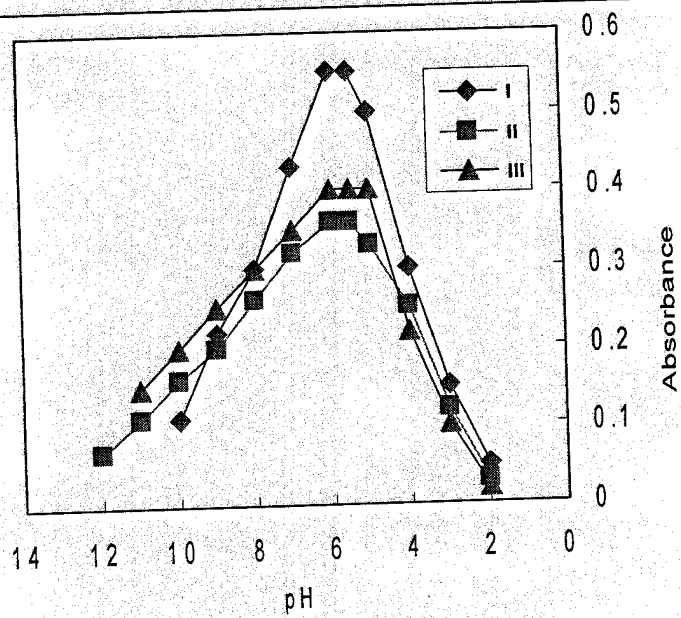


Fig. 1: Effect of pH values on the absorbance of ion pairs of Drug I, II, and III

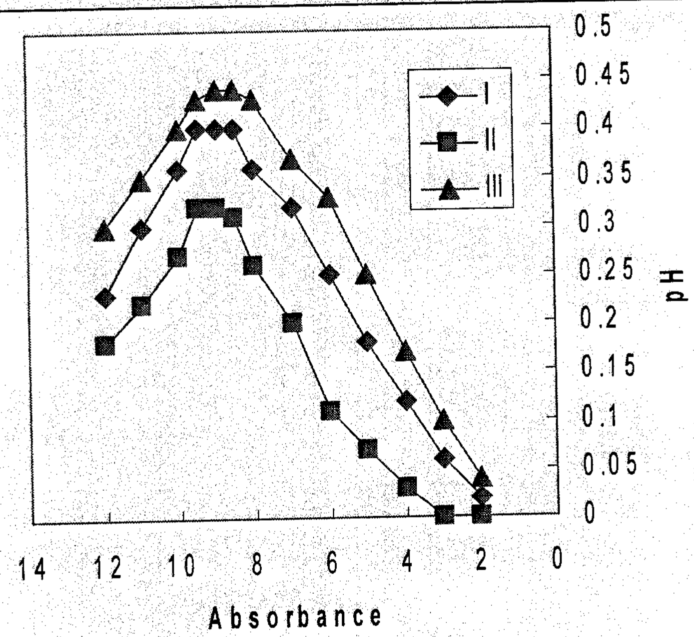


Fig. 2: Effect opf pH on the absorbance of ion pairs formed using rose bengal with drug I, II and III

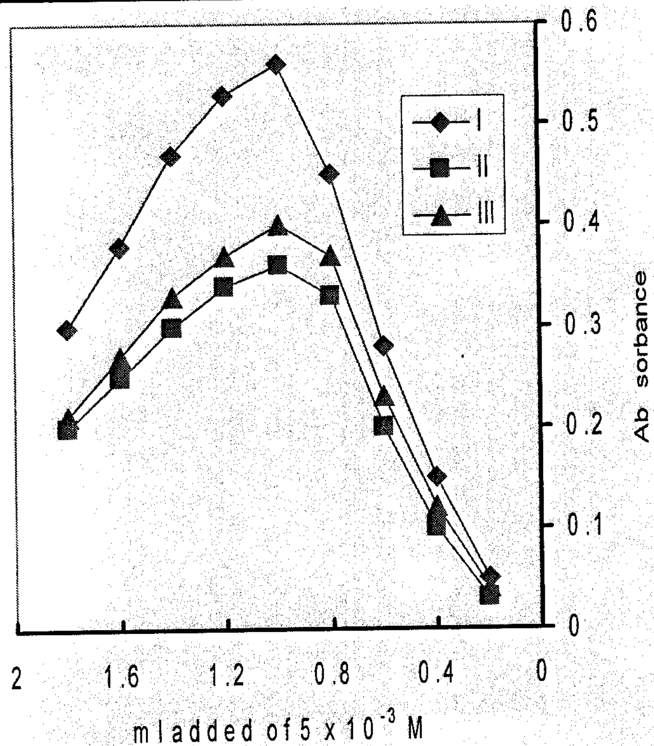


Fig 3: Effect or methyl red concentration (5×10^{-3} M) on the ion pair complex with drug I, II and III

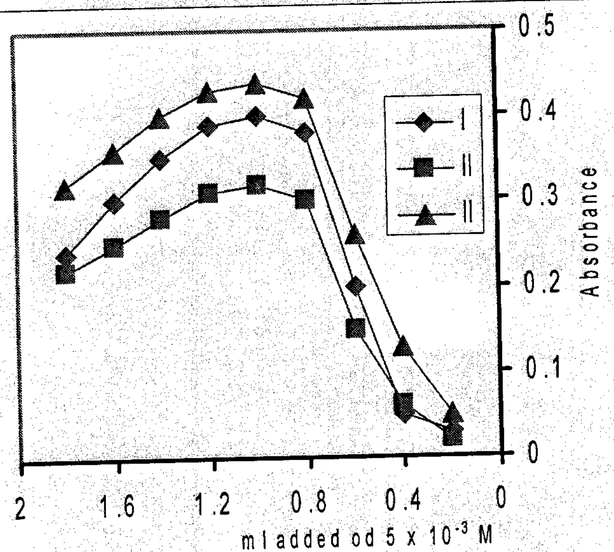


Fig 4: Effect of rose bengal concentration on absorbance of formed ion pairs with drug I, II and III